



Native Larvivorous Fishes vs Alien Fishes: A Comparative Study of Biological Control of Mosquito Larvae

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Fishes as biocontrol agents were used since past few decades. Two locally available murrels species of Assam – *Channa punctata* and *Channa stewartii* have been used to observe their larvicidal efficacy during daytime and in the evening with artificial light in three different life stages - fry, juvenile and adult after 12 and 24 hr starvation providing two densities of mosquito larvae (n=50 and n=100) and compared with the three life stages of alien fish *Poecilia reticulata* (guppy). Larvicidal efficacy of native fishes (murrel) were found significantly higher ($p < 0.05$) than the exotic ones. Among three stages of native fishes, juveniles were significantly ($p < 0.05$) more larvicidal than the exotic ones. The rate of consumption by native fish is found to increase after 24-hr starvation than 12 hr starvation. Further, fry and juveniles of murrels are more voracious predators of mosquito larvae than the exotic fishes. Small shallow, filthy water bodies are the breeding grounds of mosquitoes and these are also natural habitats of murrels. As murrels are hardy air-breathing fish, they are easy to culture in small enclosures. Use of native fish species can minimize the adverse impacts of insecticides.

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Keywords: Biocontrol; mosquito larvae; murrels; larvicidal fish; larvicidal efficacy.

1. INTRODUCTION

Mosquitoes are considered the number one public health hazard [1,2]. The use of pesticides on mosquitoes has harmful impacts on non-target organisms and leads to developing resistance in mosquitoes, which in turn aids in the resurgence of mosquito-borne ailments and this rekindled the interest in developing simple, sustainable methods [3]. Except for a few adulticides, many pesticides were banned by environmental protection agencies. There should be a new suitable, eco-friendly strategy for mosquito control [4].

Since 1900, larvivorous fishes such as *Gambusia affinis* and *Poecilia reticulata* have been in use for mosquito control. Mention of several workers may be made who used indigenous fish for mosquito control in different regions of the world [5-12]. However, the introduction of non-native fishes for mosquito control is not suitable for its invasive nature that causes ecological backlash and reduces the native aquatic flora and fauna [13,14]. The extinction of many native species remained unnoticed due to the use of *Gambusia* for mosquito control. It is important on the part of India to take a firm stand to avoid further introduction of the fish to minimize its harmful impacts [15]. The north-eastern part of India is gifted with enormous water bodies particularly floodplain rivers and adjacent wetlands which are locally called *beels*. The states of this region have uneven topography and high precipitation and are endowed with diverse aquatic habitats. The floodplain lakes are the sources of varieties of fish with huge potential for fisheries [16]. Literature on the larvicidal efficiency of native *Channa* species was found to be very scanty. Considering these aspects, a study has been made to explore the larvicidal efficacy of an easily available native murrel of Assam which also has ornamental value.

2. MATERIALS AND METHODS

2.1 Collection of Fish Specimens

Live fish specimens were procured from Guijan Ghat, Tinsukia Dist., an official fish landing centre. After procurement, the specimens were treated with 5% KMnO₄ solution before releasing into the aquaria. Collected specimens were identified following Talwar & Jhingran [17].

2.2 Measurement of Fish

Total length (cm) and total weight (g) of fish specimens were measured following [18].

2.3 Experimental Fishes

The experiment was conducted from May to September 2019. Native murels were selected for the study – a) *Channa punctata* and *Channa stewartii* were collected from the fish landing centers of Guijan, Tinsukia of upper Assam. An alien fish, guppy (*Poecilia reticulata*) was collected from an unauthorized aquarium dealer. Before the experiment, the collected specimens were dipped in 1ppm KMnO₄ solution as a prophylactic measure. Mean length (L) and weight (W) of the fry, juvenile and adult stages of selected fish species were measured from zero scale and weight with an electronic balance is given in Table 1.

2.4 About the Size of the Aquarium and Water Volume

As mentioned above, the size of the species varies widely as well as their behaviour. Adult guppy (4.2- 4.5 cm) are almost 1/3 of adult *Channa stewartii*. Further, juvenile and adult murrels are habitual jumpers and these fishes are to be kept at a lower water level.

2.5 Design of Experiments

For larvicidal efficacy, three different sizes of glass aquaria measuring 60 x 30 x 40cm, 40 x 30 x 30cm and 25 x 15 x 18cm (length x breadth x height) were used to conduct experiments on adult, juvenile and fry stages respectively. The aquaria were filled with 20-lit, 12-lit and 2-lit water from the largest to the smallest size to conduct experiments on the three life stages from adult to fry respectively during natural daylight at 9.00 am and 7.00 pm under artificial (tube) light [19]. Feeding experiments were carried out separately on the three life stages with a single fish and a group comprising three fish of all three stages with three replicates each repeated five times. The natural condition of a fish habitat was maintained [20] in the experimental aquaria. The same set of fish or groups is not repeated for the subsequent feeding trial. Feeding experiments were

Table 1. Length and weight of the fry, juvenile and adult stages of selected fish species

Fish species Size	Fry		Juvenile		Adult	
	L (cm)	W(g)	L (cm)	W(g)	L(cm)	W(g)
<i>Channa punctata</i>	2.4 ± 0.1	0.08 ± 0.01	6.7 ± 0.56	3.27 ± 0.8	13.9 ± 0.96	24.3 ± 3.5
<i>Channa stewartii</i>	2.33 ± 0.15	0.07 ± 0.01	6.53 ± 0.45	2.23 ± 0.25	14.26 ± 0.75	12.43 ± 2.85
<i>Poecilia reticulata</i>	1.0 ± 0.1	0.08 ± 0.01	2.0 ± 0.35	0.85 ± 0.04	4.3 ± 0.15	0.9 ± 0.17

monitored for 1 hour directly by close observation and then the left-out mosquito larvae were collected and counted. Mosquito larvae were collected from stagnant water bodies by a plankton net and brought to the laboratory. For regular supply, mosquito larvae culture was set up in trays and tubs filled with water a few pieces of potatoes and a small amount of cow dung [21] and was left undisturbed in dark corners to attract mosquitoes to lay eggs. Mosquito larvae were harvested with fine mesh-size nets.

Separate feeding experiments were conducted by keeping the individual fish and groups for 12 hours [22] and 24 hours [23] of starvation separately. 4th, 3rd and 2nd instar mosquito larvae were supplied separately for adult, juvenile and fry stages respectively. The adults have a large mouth gape and are not comfortable with predating smaller prey and younger fish have small mouth gapes and are unable to devour larger prey. Fishes were provided with two sets of larval densities – 50 larvae [24] and 100 larvae [21]. The temperature and pH of the water were recorded every alternate day during the trial period.

2.6 Statistical Analysis

ANOVA tests revealed significant variations in the rate of mean consumption among adult individuals of murrels (*Channa*) and guppy ($p < 0.05$). Further, the mean consumption rate by adult murrels in groups varied significantly from that of the exotic guppy ($p < 0.05$)

3. RESULTS AND DISCUSSION

Consumption efficacy of mosquito larvae by fry, juvenile and adult stages of the three experimental fishes after two different starvation periods - 12 hr and 24 hr with two different densities ($n = 50$ and $n = 100$) of mosquito larvae during the day and in night was studied individually and in groups (Table 2 and Table 3). p – values of one way ANOVA between each native fish with the exotic *Poecilia* (guppy)

individually and in group are given in the Table 2 and 3.

During the study, temperature of water was found to vary from 25.58°C – 27.8°C and pH 7.4 – 8.2. The consumption rate of mosquito larvae (density: 50 larvae) after 12 hrs starvation revealed that individual fry of *C. stewartii* and *C. punctata* could devour more larvae than that of Guppy during both day and evening. It was seen that guppy singly could devour 11 to 19 larvae in 1hr after 12 hr starvation with a density of 50 larvae.

As a whole, all the native fishes consumed more larvae /min than the exotic ones. Again, among native fishes, the juvenile stages of both *C. punctata* and *C. stewartii* were more larvorous than the exotic juveniles both at individual and group levels irrespective of starvation hours and the number of larvae supplied to them. In the case of fry and juvenile stages of native fishes, there was a significant difference ($p < 0.05$) with the same stages of *Poecilia* after both the starvation hours and two variable densities of mosquito larvae during day and night. Even in adults, there was a significant difference between the native and exotic fish. With the increase in a starvation hour and density of larvae from 50 to 100, consumption of larvae by all stages of native murrels was found to be enhanced than their exotic counterparts. It was reported that consumption of larvae increased with the increased density of larvae till the fish attained satiation [25]. Some workers also found that preference for mosquito larvae gradually diminished with the increase in size of the fish [26]. In the present study, too, adults of native fish species were found to devour less numbers of larvae than their fry or juvenile stages.

Juvenile individuals of *C. punctata* devoured highest of 54.58 ± 14.8 nos./min and *C. stewartii* 54.35 ± 6.47 nos./min after 24 hr with 100 larvae (Table 2), similarly, in the group also, the same native stage showed higher larvorous tendency in both day and night with the same density and starvation hours. Exotic guppy

**Table 2. Mean consumptionrate (nos. /min)by individual fish
(p-value in parenthesis)**

Fish species	Feeding time & interval(hrs)	Fry		Juvenile		Adult		
		n= 50	n= 100	n= 50	n= 100	n= 50	n= 100	
<i>C. punctata</i>	Day	12	0.37± 0.04 (5.31E-05)	0.47±0.03 (3.89E-10)	25.03 ± 5.72 (2.61 E- 11)	44.24 ± 5.25 (8.94E-12)	13.61± 2.7 (3.87E-08)	16.65±2.4 (8.47E-09)
		24	0.42± 0.05 (5.31E-05)	0.46±0.04 (3.64E-09)	30.97± 5.8 (5.68E-10)	54.58 ± 14.8 (1.14E-10)	14.52±3.21 (4.46E-09)	24.49± 4.6 (3.82E-09)
	Evening	12	0.35 ± 0.03 (2.43E-07)	0.43±0.03 (2.79E-09)	22.43 ± 4.3 (1.23E-08)	40.03± 6.25 (2.34E-13)	12.7± 1.9 (3.99E-08)	15.3± 1.1 (2.53E-06)
		24	0.39 ± 0.06 (2.43E-07)	0.44±0.04 (4.97E-10)	27.52 ± 7.9 (4.26E-09)	50.98 ± 12.66 (2.74E-09)	13.65±2.76 (3.6E-06)	22.93± 3.81 (3.53E-09)
<i>C. stewartii</i>	Day	12	0.39 ± 0.06 (5.37E-05)	0.41±0.04 (2.31E-07)	21.76 ± 3.08 (4.9E-10)	41.35 ± 3.38 (5.35E-13)	16.33± 2.14 (5.43E-09)	18.04±2.51 (3.89E-11)
		24	0.42± 0.07 (5.84E-07)	0.44±0.04 (3.67E-08)	23.61± 5.04 (5.05E-07)	54.35 ± 6.47 (6.01E-11)	16.91± 2.19 (4566.251)	28.15± 3.58 (7.32E-09)
	Evening	12	0.38 ±0.05 (2.5E-06)	0.38±0.36 (7.71E-07)	20.54± 2.30 (3.64E-10)	39.98± 2.83 (8.25E-12)	14.91± 1.71 (2.38E-09)	15.89±1.15 (2.05E-12)
		24	0.40 ± 0.059 (3.71E-05)	0.40±0.03 (1.69E-08)	21.68 ± 3.78 (9.01E-10)	49.93± 4.74 (1.49E-18)	15.24± 2.12 (4.27E-11)	26.65± 2.69 (8.21E-11)
<i>P. reticulata</i>	Day	12	0.25 ± 0.04	0.22±0.03	1.28 ± 0.11	0.41±0.03	3.45± 0.48	2.36± 0.22
		24	0.24± 0.04	0.23±0.03	1.33 ± 0.15	0.47±0.03	3.79 ± 0.36	2.09± 0.18
	Evening	12	0.23 ± 0.027	0.21±0.02	1.23± 0.08	0.38±0.03	3.08± 0.31	2.06 ± 0.18
		24	0.24 ± 0.04	0.21±0.03	1.35± 0.18	0.46±0.04	3.61 ± 0.23	2.27± 0.16

Table 3. Mean consumption rate (nos. /min) by group fish (p -value in parenthesis)

Fish species	Feeding time & interval (hrs)	Fry		Juvenile		Adult		
		n= 50	n= 100	n= 50	n= 100	n= 50	n= 100	
<i>C. punctata</i>	Day	12	20.47± 4.02 (1.05E-12)	2.54±0.22 (1.32E-11)	32.0± 8.18 (0.0002)	67.12± 11.94 (2.38E-12)	17.78 ± 2.89 (0.716748)	23.69 ± 4.82 (0.012906)
		24	33.09± 6.44 (1.05E-12)	2.51±0.15 (2E-11)	45.14 ± 4.8 (1E-08)	78.71 ± 9.32 (1.7E-12)	19.16 ± 3.12 (0.120985)	29.82± 7.09 (0.000366)
	Evening	12	18.91 ± 3.15 (1.32E-10)	2.49±0.18 (1.41E-10)	27.92 ± 8.45 (0.00083)	63.53 ± 11.61 (3.55E-09)	16.63 ± 4.07 (0.119627)	20.62 ± 3.37 (0.361724)
		24	30.71 ± 6.13 (1.32E-10)	2.49±0.08 (4.55E-14)	42.01 ± 6.04 (7.74E-09)	72.71 ± 10.02 (6.55E-11)	15.22 ± 4.01 (0.01909)	26.55 ± 5.66 (0.0679)
<i>C. stewartii</i>	Day	12	18.9 ± 2.57 (2.91E-10)	2.42±0.18 (1.58E-11)	32.32 ± 4.91 (5.88E-07)	63.56± 10.49 (8.21E-11)	17.6 ± 2.57 (0.662751)	27.58± 2.77 (4.93E-05)
		24	27.59 ± 6.23 (3.79E-07)	2.49±0.16 (6.13E-15)	43.27 ± 6.25 (6E-10)	75.9 ± 9.05 (2.69E-12)	18.48 ± 3.13 (0.03818)	35.85± 4.78 (2.18E-05)
	Evening	12	18.25 ± 2.12 (1.43E-12)	2.38 ± 0.16 (1.06E-10)	30.01± 4.17 (2.93E-09)	58.03± 9.35 (1.79E-08)	16.4 ± 2.20 (0.043693)	26.38 ± 2.19 (1.08E-05)
		24	26.09 ± 5.13 (4.73E-08)	2.46±0.11 (5.97E-14)	38.93± 6.67 (4.39E-06)	73.15± 7.30 (1.15E-11)	16.08± 2.55 (0.01981)	32.66± 4.23 (3.17E-06)
<i>P. reticulata</i>	Day	12	1.12 ± 0.12	0.40±0.04	13.10 ± 1.01	2.36±0.23	18.36 ± 4.19	18.93± 3.45
		24	1.19 ± 0.11	0.39±0.04	18.35 ± 1.78	2.41±0.16	22.94 ± 4.58	24.69 ± 2.19
	Evening	12	1.09 ± 0.09	0.38±0.04	12.68 ± 1.05	2.36±0.20	19.73 ± 4.07	19.68 ± 2.17
		24	1.17± 0.1	0.36±0.04	17.23 ± 2.00	2.39±0.18	19.85 ± 3.38	23.47 ± 2.73

revealed increased consumption in the adult stage only that too, much lower nos./min than the native fishes. It was reported that guppies could predate sufficient numbers of mosquito larvae with their increase in body size [27]. Alien fishes were found to be more larvicidal with the increase in their size. Adults were more predatory in comparison to the fry and juvenile stages. However, the rate of predation of native fishes was significantly ($p < 0.05$) better than the alien fish. Thus, exotic species in the adult stage and murrels in their juvenile stage were found more effective in controlling mosquito larvae as also pointed out by Gogoi [28] in an earlier experiment with a combination of six native and alien species.

Mean consumption nos./min during day and night was found to differ markedly, predation during the day was higher than the night time. *Channa gachua* was also found to be a diurnal predator, the fish predated more during morning time [29]. Investigation on the consumption of *Culex* larvae by the fish *Trichopodus trichopterus* found that diurnal predation was more than nocturnal one and reported that visual cues are important for the consumption of prey [30]. Several workers reported that feeding deprivation in fish leads to intense feeding [31-33]. The biocontrol approach using larvivorous fish has been eco-friendly, cheap, effective and safe for human beings and non-target organisms [34,35]. For biocontrol of mosquitoes, exotic fishes like *Gambusia* and *Poecilia* were often recommended. However, the use of native fishes is recommended to get rid of the harmful impacts of exotic fishes like *Gambusia* and *Poecilia* [36].

4. CONCLUSION

The rate of consumption of mosquito larvae by two experimental groups was different – the smaller size group both at individual and group levels revealed more consumption of larvae. It showed variable amount of consumption of larvae between day and night and between interval of feeding time. Alien fishes were found to be more larvicidal with the increase in their size. Adults were more predatory in comparison to fry and juvenile stages. However, the rate of predation of native was significantly ($p < 0.05$) better than the alien fish.

As a whole, native fry and juveniles exhibited significantly ($p < 0.05$) higher predation rate than those of exotic fish. Both the native murrels are available in the wetlands of the NE

regions. They are now threatened due to anthropogenic activities like indiscriminate fishing for commercial purpose. The breeding grounds of mosquitoes are also the habitats of murrels as they can live in filthy water bodies and can tolerate higher water temperature, low levels of dissolved oxygen. Proper culture of murrels can develop sustainable source of these larvicidal fishes as better alternatives to exotic fishes and harmful chemical pesticides.

Indigenous fishes are more adapted under the prevailing conditions. They are more tolerant to the seasonal changes in the habitat. Mosquito larvae are the natural food of these fish species at least in their fry and fingerling stages. They breed naturally and unlike exotic fishes do not cause harm to other native fishes. Hence, there is ample scope for investigation on indigenous fishes like murrels than the exotic guppy to use them as the biological control agent.

ETHICAL APPROVAL

The ethical approval in the present case is not mandatory. The experimental fish species are also considered as aquarium fish and these are procured from fish dealers who are license holders. The species were reared just like aquarium species and the specimens were fed mosquito larvae only, instead of commercial feed. Moreover, no specimens were killed during the experiment. 100% survival was recorded during the feeding trial and after the experiment, all the live specimens were released in their natural habitat.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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